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FINAL REPORT
NAVY CONTRACT No. N00014-86-K-0347
Fred H. Gage, Principal Investigator

During the three years of the contract for the Office of Naval Research we have published 22 articles in referred Journals and 8 chapters which summarize the research that was conducted in part through this contract. A listed of these publications is attached to this report.

I have summarized four articles which capture some of our main accomplishments and which provide a basis for much of the work presently underway.

1. Davis, G.E., Blaker, S.N., Engvall, E., Varon, S., Manthorpe, M., and Gage, F.H. *Human Amnion membrane serves as a substratum for growing axons in vitro and in vivo.* Science, 236:421-436, 1987.

The epithelial cell layer of human amnion membrane can be removed while the basement membrane and stromal surfaces remain morphologically intact. Such a preparation has been used as a substratum for the in vitro culture of dissociated neurons. Embryonic motor neurons from chick ciliary ganglion attached to both surfaces but grew extensive neurites only on the basement membrane. On cross sections of rolled amnion membranes, regenerating axons of cultured neurons were guided along pathways of basement membrane that were immunoreactive with an antibody to laminin. In addition, when rolled amnion membranes were implanted into a lesion cavity between the rat septum and hippocampus, cholinergic neurons extended axons through the longitudinal oriented implant into the hippocampus. Thus, this amnion preparation can serve as a bridge to promote axonal regeneration in vivo in damaged adult brain.

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2. Rosenberg, M.B., Friedmann, T., Robertson, R.C., Tuszynski, M., Wolff, J.A., Breakefield, X.O., and Gage, F.H. *Grafting genetically modified cells to the damaged brain: Restorative effects of NGF expression.* Science, 242: 1575-1578, 1988.

Fibroblasts were genetically modified to secrete nerve growth factor (NGF) by infection with a retroviral vector and then implanted into the brains of rats that had surgical lesions of the fimbria-fornix. The grafted cells survived and produced sufficient NGF to prevent the degeneration of cholinergic neurons that would die without treatment. In addition, the protected cholinergic cells sprouted axons that projected in the direction of the cellular source of NGF. These results indicate that a combination of gene transfer and intracerebral grafting may provide an effective treatment for some disorders of the central nervous system.

3. Gage, F.H., Olejniczak, P., and Armstrong, D.M. *Astrocytes are important for sprouting in the septohippocampal circuit.* Experimental Neurology, 102: 2-13, 1988.

Damage to the fimbria-fornix, and separately to the perforant path, leads to distinct and dramatic time-dependent increases in glial fibrillary acidic protein immunoreactivity (GFAP-IR) in specific areas of the hippocampal formation. Specifically, fimbria- fornix lesions resulted in an increase in the GFAP-IR in the pyramidal and oriens area of the CA3 as well as the inner molecular layer of the dentate gyrus. In addition, in the septum ipsilateral to the lesion, there was a rapid and robust increase in GFAP-IR in the dorsal lateral quadrant of the septum, but not in the medial region. Only after 30 days did the GFAP-IR reach the medial septum. Following perforant path lesions, there was a selective increase in GFAP-IR in the outer molecular layer of the dentate gyrus. Most of these changes were transient and had disappeared by 30 days postlesion. We speculate that the increase in GFAP-IR in these target areas is a necessary requirement for the sprouting responses that are observed. This hypothesis is supported by the fact that astrocytes secrete NGF in vitro and that NGF activity increases in these target areas following these same lesions. A mechanism for the selective activation of the astrocytes through the initial activation of microglia and secretion of interleukin-1 is postulated.

4. Gage, F.H., Batchelor, P., Chen, K.S., Chin, D., Higgins, G.A., Koh, AS., Deputy, S., Rosenberg, M.B., Fischer, W., and Bjorklund, A. *NGF receptor re-expression and NGF mediated cholinergic neuronal hypertrophy in the damaged adult neostriatum.* Neuron, 2: 1177-1184, 1989.

Adult cholinergic interneurons of the neostriatum are not immunoreactive for monoclonal antibody to NGF receptor, whereas the developing neostriatum is immunoreactive for this same antibody. Chronic NGF infusion into the adult neostriatum resulted in reexpression of the NGF receptor such that many cholinergic interneurons became immunoreactive for NGF receptor. NGF infusion dramatically increased the size and choline acetyltransferase immunoreactivity of these same cholinergic neurons. Additionally, in situ hybridization demonstrated an increase in the number of cells expressing NGF receptor mRNA in the NGF-infused striatum. These findings indicate that central cholinergic neurons which lose their NGF receptors during postnatal development will resume their NGF responsiveness when the tissue is damaged. Such a damage-induced mechanism may act to enhance the action of trophic factors, including NGF, released at the site of injury, and enhance the responsiveness of damaged CNS neurons to exogenously administered trophic factors.

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Office of Naval Research Publication List

1. Kesslak, J.P., Frederickson, C.J., and Gage, F.H. Quantification of hippocampal noradrenaline and zinc changes after selective cell destruction. Experimental Brain Research, 67:77-84, 1987.

2. Peterson, G.M., Williams, L.R., Varon, S., and Gage, F.H. Loss of GABAergic neurons in medial septum after fimbria-fornix transection. Neuroscience Letters 76:140-144, 1987.

3. Davis, G.E., Blaker, S.N., Engvall, E., Varon, S., Manthorpe, M., and Gage, F.H. Human amnion membrane serves as a substratum for growing axons in vitro and in vivo. Science 236:1106-1108, 1987.

4. Shults, C.W., Johnston, P., and Gage, F.H. Comparison of substance K-like and substance P-like fibers and cells in the rat hippocampus. Brain Research, 426:290-296, 1987.

5. Armstrong, D.M., Bruce, G., Hersh, L.B., and Gage, F.H. Development of cholinergic neurons in the septal/diagonal band complex of the rat. Developmental Brain Research, 36: 249-256, 1987.

6. Gage, F.H., Armstrong, D.M., Williams, L.R., and Varon, S. Morphological response of axotomized septal neurons to nerve growth factor. Journal of Comparative Neurology, 269: 147-155,1988.

7. Gage, F.H., Brundin, P., Strecker, R., Dunnett, S.B., Isacson, O., and Bjorklund, A. Intracerebral neuronal grafting in experimental animal models of age-related motor dysfunction. N.Y. Academy of Science, 515: 383-395, 1988.

8. Blaker, S.N., Armstrong, D.M., and Gage, F.H. Cholinergic neurons within the rat hippocampus: Response to fimbria-fornix transection. Journal of Comparative Neurology, 272:127-138, 1988.

9. Gage, F.H. and Varon, S. Trophic hypothesis of neuronal cell death and survival. In: Recovery of function after brain damage. Eds. Finger, et. al., pp. 201-214, 1988.

10. Armstrong, D.M., Hersh, L.B., and Gage, F.H. Morphologic alterations of cholinergic processes in the neocortex of aged rats. Neurobiology of Aging, 9:199-205, 1988.

11. Buzsaki, G., Freund, T., Bjorklund, A., and Gage, F.H. Restoration and deterioration of function by brain grafts in the septohippocampal system. Progress in Brain Research, D.M. Gash and J.R. Sladek, Jr. (Eds.) 78:69-77, 1988.

12. Gage, F.H., Blaker, S.N., Davis, G.E., Engvall, E., Varon, S., and Manthorpe, M. Human amnion membrane matrix as a substratum for axonal regeneration in the central nervous system, Experimental Brain Research, 72: 371-380, 1988.

13. Buzsaki, G., Bickford, R.G., Ponomareff, G., Thal, L.J., Mandel, R., and Gage, F.H. Nucleus basalis and thalamic control of neocortical electrical activity in the freely moving rat. Journal of Neuroscience, 8(11): 4007-4027, 1988.

14. Gage, F.H., Chen, K.S., Buzsaki, G., and Armstrong, D. Experimental approaches to age related cognitive impairments. Neurobiology of Aging, 9: 645-655, 1988.

15. Buzsaki, G., Ponomareff, G., Bayardo, F., Shaw, T., and Gage, F.H. Suppression and induction of epileptic activity by neuronal grafts. PNAS, 85:9327-9330, 1988.

16. Gage, F.H., Olejniczak, P., and Armstrong, D.M. Astrocytes are important for sprouting in the septo-hippocampal circuit. Experimental Neurology, 102: 2-13, 1988.

17. Gage, F.H., Wolff, J.A., Rosenberg, M.B., Xu, L., Yee, J.-K., Shults, C., and Friedmann, T. Implantation of genetically engineered cells to the brain. Progress in Brain Research, D.M. Gash and J.R. Sladek, Jr. (Eds.), 78:651-658, 1988.

18. Rosenberg, M.B., Friedmann, T., Robertson, R.C., Tuszynski, M., Wolff, J.A., Breakefield, X.O., and Gage, F.H. Grafting of genetically modified cells to the damaged brain: Restorative effects of NGF gene expression. Science, 242: 1575-1578, 1988.

19. Buzsaki, G. and Gage, F.H. Absence of long-term potentiation in the subcortically deafferented dentate gyrus. Brain Research, 484: 94-101, 1989.

20. Gage, F.H., Batchelor, P., Chen, K.S., Chin, D., Higgins, G.A., Koh, S., Deputy, S., Rosenberg, M.B., Fischer, W., and Bjorklund, A. NGF receptor re-expression and NGF mediated cholinergic neuronal hypertrophy in the damaged adult neostriatum. Neuron, 2:1177-1184, 1989.

21. Batchelor, P.E., Armstrong, D.M., Blaker, S.N., and Gage, F.H. Nerve growth factor receptor and choline acetyltransferase co-localization in neuron s within the rat forebrain: Response to fimbria-fornix transection. Journal of Comparative Neurology, in press, 1989.

22. Gage, F.H., and Buzsaki, G. CNS grafting: Potential mechanisms of action. Neural Regeneration and Transplantation, 1989, Alan R. Liss, Inc., pp. 211-226.

23. Shimohama, S., Rosenberg, M.B., Fagan, A.M., Wolff, J.A., Short, M.P., Breakefield, X.P., Friedmann, T., and Gage, F.H. Grafting genetically modified cells into the rat brain: Characteristics of E.coli ß-galactosidase as a reporter gene. Molecular Brain Research, 5: 271-278.

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28. Gage, F.H., and Buzsaki, G. Experimental therapeutic approaches: Intracerebral grafting and neurotrophic factors. In Alzheimer's Disease: Treatment and Long-Term Management by Cummings and Miller, pp. 353-370, 1989.

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30. Gage, F.H., and Buzsaki, G. Neuronal grafting to the damaged adult hippocampal formation. In The Hippocampus - New Vistas, pp. 235-253, Alan R. Liss, Inc., 1989.